Microbial Iron Chelators: Total Synthesis of Aerobactin and Its Constituent Amino Acid, N^6-Acetyl-N^6-hydroxylysine

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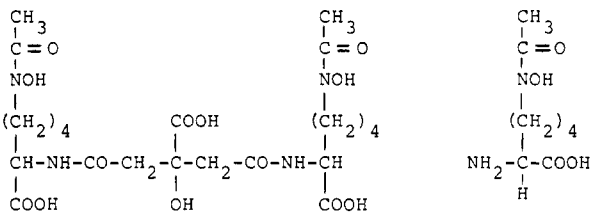
Abstract: The synthesis of the natural ferric ionophore (−)-aerobactin (**1**) and of its constituent amino acid N^6-acetyl-N^6-hydroxylysine (**2**) is described. The benzyl hydroxamates **8** and **9** were subjected to triphenylphosphine–diethyl azodicarboxylate-mediated alkylations with the ε-hydroxynorleucine derivative **7a**. While **8** gave a complex mixture with predominant carbonyl O-alkylation, **9** was cleanly N-alkylated to yield **14**. Compounds **10a,b** were prepared by direct alkylation of **8** with bromides **15a,b**, which gave predominant N-alkylation. Hydrogenation of (D,L)-**10a** yielded (D,L)-N^6-acetyl-N^6-hydroxylysine (**2**). Optically active **10b**, prepared from (L)-ε-hydroxynorleucine, was α-N deprotected and coupled with anhydromethylenecitryl chloride (**18**) to yield **19**, which was deprotected in two steps to yield (−)-aerobactin (**1**).

Correct physiological iron balance is essential for the proper functioning of the enzymes and pigments that facilitate electron transport, oxygen transport, and other life-sustaining processes. Although iron is one of the most abundant elements, its extreme water and membrane insolubility make its assimilation and regulation by organisms, including humans, difficult. Thus, iron deficiency and overload are common and significant health problems. Most individuals with iron deficiency can be successfully treated with frequent doses of ferrous salts. However, disorders such as Cooley's anemia in which the transfusional treatment results in increased body iron are more serious. Extensive iron overload causes deposition of the metal in a number of organs, causing tissue damage and early death.[1] Treatment of such genetic problems includes the development of efficient iron chelators. The design of such drugs may be greatly aided by consideration of the naturally occurring iron chelators. Microbial systems have evolved highly specific and efficient iron-sequestering agents.[2-4] These natural iron chelators or siderophores consist of two main chemical classes: the hydroxamic acids[3-6] and the catecholes.[6,7] Considerable effort has recently been devoted to the synthesis and study of natural hydroxamate and catecholate siderophores and analogues of potential medicinal use.[8]

Nature has taken advantage of the ω-amino groups of ornithine and lysine to construct secondary hydroxamic acid residues found in siderophores.[3-6] Thus, the key to the synthesis of most of the hydroxamate-containing siderophores is the preparation of the constituent ω-N-hydroxy amino acids in optically pure form. N^5-hydroxyornithine is a constituent of rhodotorulic acid,[9] ferrichrome,[10,11] ferrichrome A,[12,13] ferrichrome C,[14] albomycins δ_1, δ_2, and ε,[5,15,16] ferrichrocin,[17] ferrichrysin,[17,18] ferrirubin and ferrirhodin,[19] fusarinine,[20] fusarinine B and C,[21] fusigen,[22] dimeric acid,[23] coprogen,[24,25] coprogen B,[23] and ferribactin.[26] Rhodotorulic acid,[27,28] dimeric acid,[28] and ferrichrome[29,30] have been synthesized. N^6-Hydroxylysine has been found only in aerobactin (**1**),[31] the mycobactins,[32] and the exochelins,[33,34] none of which have previously been synthesized. Aerobactin and the mycobactins have been the targets of recent synthetic studies in our laboratory. Our successful synthesis of (−)-cobactin T[35] (a major saponification product of mycobactin T, which contains N^6-hydroxylysine in a seven-membered lactam ring) and aerobactin, reported herein, marks the first synthetic entries into this class of siderophores.

Aerobactin (**1**) consists of two residues of N^6-acetyl-N^6-hydroxy-(L)-lysine (**2**) linked to a central residue of citric acid by amide bonds.[31,36] The two terminal carboxyl groups of citric acid are coupled to the α-amino group of the amino acid. The internal citrate carboxyl and the α-carboxyls of the amino acid residues are free. Thus, with the hydroxamic acid moieties, aerobactin contains five titrable protons. The N^6-acetyl-N^6-hydroxylysine fragments constitute the main challenge in the synthesis of aerobactin.



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† Fellow of the Alfred P. Sloan Foundation, 1981–1983.

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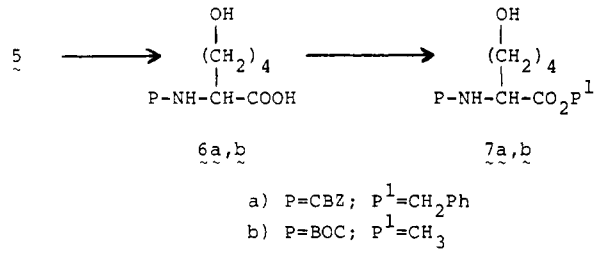
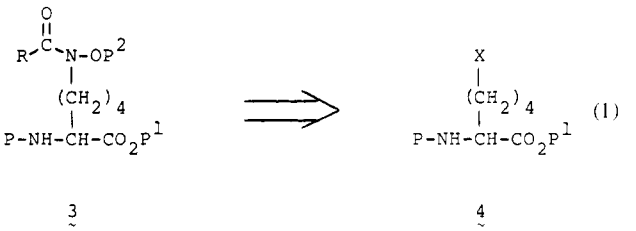
Scheme I



Considerable attention has been given to the synthesis of N^5-hydroxyornithine and its derivatives. Neilands' group first prepared N^5-hydroxyornithine as the noncrystalline dihydrochloride and as the crystalline 2-nitro-1,3-indandione salt.[13,37] They converted 3-bromopropylhydantoin to the nitro compound, reduced to the hydroxylamine, and opened the hydantoin ring by acid hydrolysis. The same scheme was used to prepare the corresponding salts of N^6-hydroxylysine.[37] Japanese chemists have synthesized N^5-hydroxyornithine·HCl by alkylating diethyl acetamidomalonate with 1-*N*-tosyl-(*N*-(benzyloxy)amino)-3-bromopropane followed by decarboxylation, hydrolysis, and hydrogenolysis.[38,39] They also reported the selective N^5-acetylation of N^5-(benzyloxy)ornithine monohydrobromide to give, after reductive debenzylation, N^5-acetyl-N^5-hydroxyornithine. The same group then incorporated enzymatically resolved N^5-tosyl-N^5-(benzyloxy)-(L)-ornithine into a diketopiperazine, which was subsequently converted to rhodotorulic acid[27] and into a cyclic hexapeptide ferrichrome precursor.[30] Swiss chemists converted ornithine to an α-protected N^5-hydroxyornithine derivative by oxidizing the corresponding N^5-benzylidene derivative and hydrolyzing the oxaziridine thus formed.[40] The same group had earlier prepared several derivatives of δ-nitronorvaline,[28,29,41] including the free amino acid, the N^2-acetyl derivative, the hydantoin, the diketopiperazine, and the cyclic hexapeptide: cyclo[(L)-δ-nitronorvaline)$_3$(glycine)$_3$]. Reduction and acetylation of the latter two compounds gave rhodotorulic acid[28] and ferrichrome,[29] respectively.

In contrast to N^5-hydroxyornithine, N^6-hydroxylysine and its derivatives have received very little synthetic attention. As mentioned above, Neilands' group prepared the noncrystalline dihydrochloride and the crystalline 2-nitro-1,3-indandione salt of the parent amino acid.[37] Australian chemists refluxed 10 mg of diethyl (*Z*)-5-(benzylidenamino)-1-(phthalimido)pentane-1,1-dicarboxylate *N*-oxide (obtained from diethyl 5-bromo-1-(phthalimido)pentane-1,1-dicarboxylate and the sodium salt of (*Z*)-benzaldoxime) with dilute HCl to obtain a water-soluble product, which gave a red color with alkaline triphenyltetrazolium chloride, and a dark color with ninhydrin, and which lacked phthalimide and ethyl ester ^{1}H NMR signals. On this basis, the product was suggested to be N^6-hydroxylysine dihydrochloride.[42] A shorter hydrolysis time apparently left the phthalimido group and ethyl esters intact and the hydroxylamino function could be N,O-diacetylated.

Unfortunately these methods do not yield derivatives that are well suited for incorporation into natural products. Such endeavors call for selectively removable protecting groups on the α-amino, the α-carboxyl group, and the hydroxamate oxygen. Such a derivative is represented by general structures **3**, where P, P^1, and P^2 can each be independently removed under mild conditions. Harsh reaction conditions are therefore incompatible with the synthesis of compounds such as **3**. A mild procedure that would introduce the O-protected hydroxamate functionality of **3** into the precursor **4** seemed ideal (eq 1). The indicated reaction is



a nucleophilic displacement of the leaving group, X, by the O-substituted hydroxamate anion: R–CO–N̄–OP^2.

The alkylation of O-substituted hydroxamates may take place on the carbonyl oxygen as well as on the nitrogen.[43] Such O-alkylations yield hydroximates that may be either *E* or *Z* in configuration. Thus, three different products may result from the alkylation. Control over this feature is most important for preparative work, and a minimum requirement of predominant N-alkylation was deemed essential. The literature indicates[43] that the outcome of the alkylation can be influenced by the following: (a) the nature of the hydroxamate O-substituent, (b) the nature of the leaving group on the alkylating agent, (c) the counterion to the hydroxamate anion, (d) the solvent, and (e) the nature of the acyl group of the hydroxamate.

In this work, the benzyl group was chosen for the hydroxamate O-substituent for reasons of stability, ease of removal, and commercial availability of O-benzylhydroxylamine. The choice of the leaving group (X in **4**) was greatly influenced by the desire to generate **4** from the corresponding alcohol (**4**, X = OH) under mild, essentially neutral, conditions. The corresponding unprotected amino acid alcohol **5**, ϵ-hydroxynorleucine, can be conveniently synthesized[44] and resolved[45,46] in 100-g quantities. The following two possibilities were considered: (a) prior conversion of the hydroxyl group of **4** (X = OH) to a halide followed by alkylation (the reaction of silver salts of hydroxamates with alkyl halides reportedly encourages carbonyl O-alkylation, but sodium and potassium salts favor *N*-alkylation[43]) and (b) direct triphenylphosphine (PPh_3)/diethyl azodicarboxylate (DEAD)-mediated alkylation of the hydroxamate with the alcohol **4** (X = OH). On the basis of work from this laboratory, it was known that by treating a mixture of an alcohol and an O-substituted hydroxamate with PPh_3 and DEAD, the hydroxamate was effectively alkylated by the alcohol.[35,47,48] When intramolecular, this procedure gave predominant N-alkylation. Finally, the use of an alkoxycarbonyl group as the acyl group of the hydroxamate was investigated because of reports of specific N-alkylation of these carbamates.[49] However, this benefit must be weighed against the necessity of eventually removing this group and replacing it with the acyl group of the synthetic target.

In addition to the amino acid hydroxamates, a citric acid synthon was required which was activated at both terminal carboxyl groups and protected at the internal carboxyl and hydroxyl

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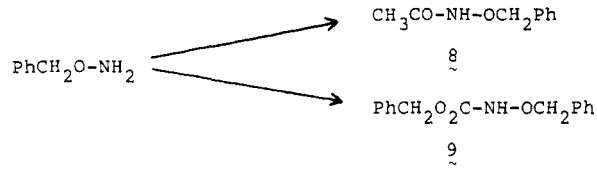
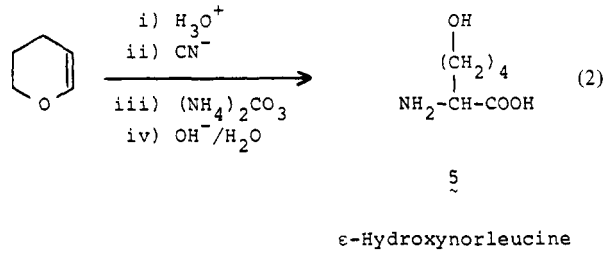
Scheme II



groups. Coupling of such a synthon with an α-N-deprotected form of **3** followed by complete deprotection should give aerobactin (**1**).

Results and Discussion

ϵ-Hydroxynorleucine and α-Protected Derivatives. The amino acid ϵ-hydroxynorleucine (**5**) was prepared by the method of Gaudry[44] (eq 2). As already indicated, the synthesis begins with



(2)

inexpensive dihydropyran and is convenient on a 100-g scale. Enzymatic resolution[46] provided the pure (L) component in 73% yield. The α-amino group was easily protected as the carbobenzoxy (Cbz) or *tert*-butoxycarbonyl (Boc) derivatives (Scheme I). For the Cbz derivative, the amino acid was treated with Cbz-Cl in aqueous $HCO_3^-CO_3^{2-}$ buffer, affording the product, **6a**, in a recrystallized yield of 83%. The Boc derivative, **6b**, was formed by treating **5** with 1 equiv of triethylamine followed by a slight excess of di-*tert*-butyl dicarbonate in H_2O–THF (1:1). These α-N-protected compounds were then esterified at the carboxyl groups. The Cbz derivative was converted to the benzyl ester, **7a**, so that both groups could later be simultaneously hydrogenolyzed. On the other hand, the Boc derivative was converted to the methyl ester, **7b**, so that either group could be independently removed. Thus, the two compounds serve in complementary capacities. The esters were formed in good yields by treating the acids with the corresponding O-alkyl-*N*,*N*′-diisopropylisourea[50] either in acetonitrile at room temperature or in refluxing methylene chloride for the benzyl ester, **7a**, and the methyl ester, **7b**, respectively. Chromatographic purification provided the benzyl ester in 87% yield. Chromatography of the more polar methyl ester was not as successful since it coeluted with impurities. Thus, only a 32% yield of pure material was obtained. However, the crude product, suitable for further use, could be obtained in over 85% yield by simply chilling the reaction mixture to crystallize out the *N*,*N*′-diisopropylurea, filtering the solution, and removing the solvent.

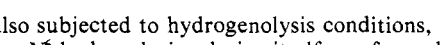
Primary *O*-Benzyl Hydroxamates. The primary *O*-benzyl hydroxamates were prepared by the direct acylation of *O*-benzylhydroxylamine (Scheme II). In this manner, *O*-benzyl acetohydroxamate (**8**) has been previously prepared.[51] *O*-Benzyl-*N*-Cbz-hydroxylamine (**9**) was prepared by the acylation of O-benzylhydroxylamine with carbobenzoxy chloride.

Hydroxamate Alkylations. A one-step procedure for converting the alcohols, **7a,b** to the hydroxamates, **10a,b**, seemed very attractive initially. As previously mentioned, PPh_3/DEAD-mediated alkylations of O-substituted hydroxamates with alcohols were known to proceed with specific[47,48] or selective[35] N-alkylation in intramolecular reactions yielding 4- or 7-membered rings, respectively. Unfortunately, when a mixture of *O*-benzyl acetohydroxamate (**8**) and an alcohol, such as **7a**, was treated with PPh_3 and DEAD, predominant O-alkylation took place (Scheme III). The main products were hydroximates **11a** and **12a**; some of the hydroxamate **10a** was formed, and a fourth product (apparently with structure **13**) was also formed, although in low yield. Such hydrazine-derived products have been previously reported.[52,53] Thus, the intermolecular alkylations proceed with reversed selectivity (O-alkylation) compared with the intramolecular alkylations, especially those forming small rings. Since we had set the goal of predominant N-alkylation, this reaction was deemed preparatively unsatisfactory.

An alternative was devised that eliminated competitive O-alkylation but which requires a subsequent acyl group replacement. As discussed above, O-benzyl acetohydroxamate is predominately O-alkylated by the alcohol/PPh_3/DEAD reagent combination. However, the O-benzyl hydroxamates bearing an *N*-alkoxycarbonyl group rather than the usual *N*-acyl group are exclusively N-alkylated by the same reagents. Thus, a mixture of an alcohol (**7a**) and *O*-benzyl-*N*-Cbz-hydroxylamine (**9**) was treated with PPh_3/DEAD, and compound **14** was obtained as the only product in 60–70% yield after chromatographic purification. Although, in this case, the N^6-Cbz group is not selectively removable, other alkoxycarbonyl groups should be selectively removable, thus enabling a subsequent N^6-acyl group replacement to give the desired hydroxamates such as **10**.

A third alkylation procedure was investigated with the hope of obtaining the N^6-acetyl-N^6-benzyloxylysine derivatives, **10a,b**, directly, without need of an acyl group replacement, and with the desired predominant N-alkylation. First, the alcohols, **7a,b**, were converted to the corresponding bromides, **15a,b**, by treatment with PPh_3 and CBr_4 in dry THF or acetonitrile at room temperature (Scheme IV). These bromides were easily purified by silica gel chromatography and obtained in high yields (85–95%). The alkylations were accomplished by refluxing the bromides with O-benzyl acetohydroxamate in dry acetone with an excess of K_2CO_3. A catalytic amount (0.1–0.5 equiv) of KI accelerated the reaction. However, use of KI resulted in some formation of the *Z* isomers of the hydroximates (**12a,b**), whereas apparently only the *E* isomers **11a,b** form in the absence of KI. Although the total amount of O-alkylation is not increased, the (*Z*)-hydroximates are more difficult to chromatographically separate from the desired products than are the (*E*)-hydroximates. [The geometrical (*E*,*Z*) assignments were tentatively made by ^{1}H NMR and chromatographic elution differences which are consistent with those of other hydroximate isomers previously reported.][43] In either case, the yield of the desired hydroxamate (**10**) was about 65% (isolated), and the hydroximates were obtained in about 13% total yield. Thus, a 5:1 ratio of N- to O-alkylation was obtained. This alkylation appears to be the most efficient method of introducing the *N*-hydroxy-*N*-acylamino group into the natural product synthons required for hydroxamate siderophore syntheses.

Deprotections Giving Free Amino Acids. The amino acid N^6-acetyl-N^6-hydroxylysine (**2**) cannot be obtained from natural aerobactin since the acetyl group is removed during hydrolysis. However, mild hydrogenolysis of N^6-acetyl-N^6-benzyloxy-N^2-Cbz-(D,L)-lysine benzyl ester (**10a**) provided the (D,L)-amino acid in good yield (eq 3). N^6-Benzyloxy-N^2,N^6-$(Cbz)_2$-lysine benzyl



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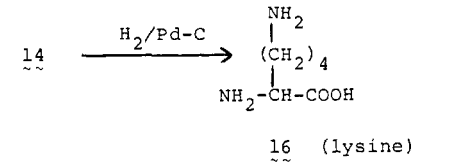
ester (**14**) was also subjected to hydrogenolysis conditions, but instead of yielding N^6-hydroxylysine, lysine itself was formed by reduction of the N–O bond (eq 4). Thus, while hydrogenolysis



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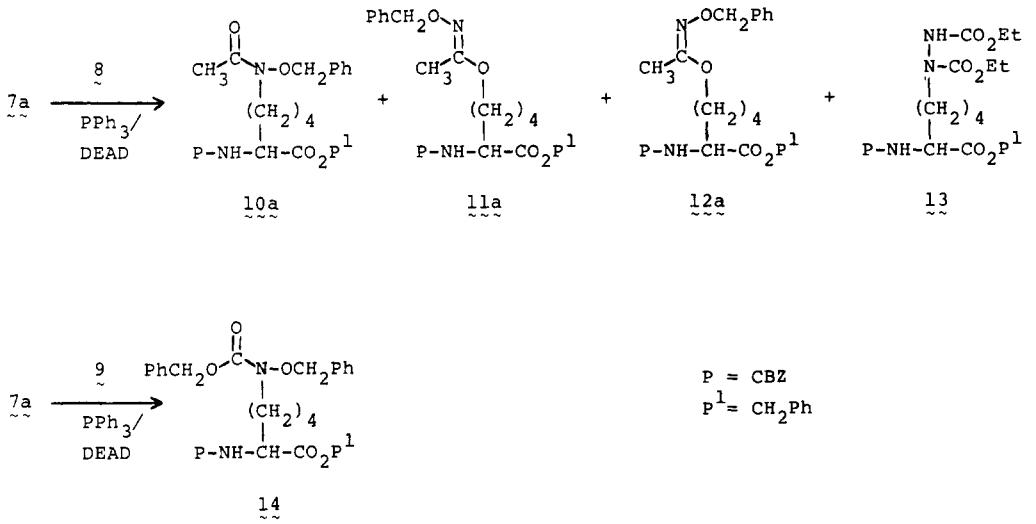
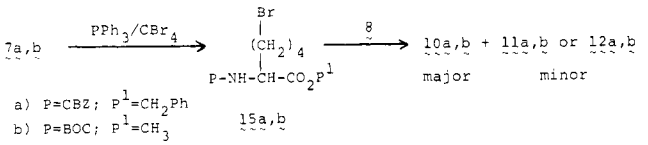
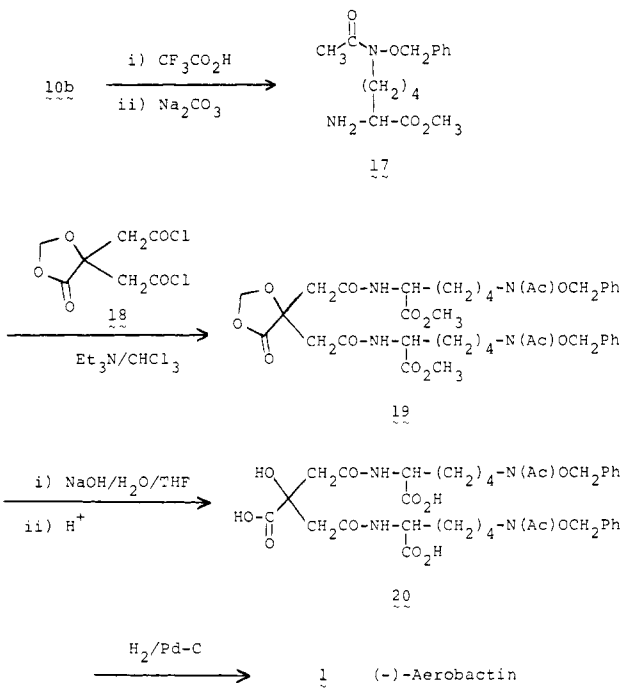
Scheme III



Scheme IV



Scheme V



is suitable for the reductive debenzylation of benzyl hydroxamates, *N*-benzyloxyamines are overreduced to the amines. Reduction of the N–O bond was previously reported during attempted hydrogenolysis of *O*-alkyl-*N*-Cbz-hydroxylamines.[54]

Synthesis of (−)-Aerobactin. For the synthesis of aerobactin, the α-amino group of a derivative such as (L)-**10** must be selectively deprotected and subsequently coupled to the two terminal carboxyl groups of citric acid. Selective α-N-deprotection was most easily accomplished with derivative **10b** by brief treatment with neat CF_3CO_2H (Scheme V). The free amine was coupled with 0.5 equiv of anhydromethylenecitryl chloride (**18**)[55] to provide an 83% yield of **19**. Deprotection was accomplished in two steps. First the two methyl esters and the methylene bridge were hydrolyzed with NaOH in THF–H_2O to provide **20** in excellent yield. Finally, the two benzyl groups were removed by hydrogenolysis to yield (−)-aerobactin (**1**) in 90–93% yield. The overall yield from (L)-ε-hydroxynorleucine was 26%.

Conclusions

The alkylation of O-substituted hydroxamates offers an attractive route for the preparation of amino acid precursors to the natural hydroxamate siderophores. The alkylations can be performed under conditions compatible with chirality and selectively removable α-N- and α-C-protecting groups, thus yielding very versatile intermediates. The use of such intermediates has been demonstrated by the total synthesis of the natural ferric ionophore (−)-aerobactin (**1**). These intermediates are also being used for the total synthesis of the mycobactins, which will be reported in due course.

Experimental Section

General Methods. Melting points were taken on a Thomas-Hoover Capillary melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 727B spectrophotometer. Proton NMR spectra were obtained on a Varian A60, EM-390, or XL-100 spectrometer in deuteriochloroform (unless otherwise stated) and are reported in parts per million downfield of internal tetramethylsilane (δ units). Optical rotations were measured with a Rudolf and Sons polarimeter No. 574. Field desorption mass spectra were obtained by John L. Occolwitz (Eli Lilly Co.).

ε-Hydroxynorleucine (5). This amino acid was prepared by the method of Gaudry[44] and resolved by the method of Bodansky et al.[35,46]

(D,L)-Cbz-ε-Hydroxynorleucine (6a). (D,L)-ε-Hydroxynorleucine (14.4 g, 0.0982 mol) was dissolved in saturated aqueous $NaHCO_3$ (250 mL) and the solution adjusted to pH 8.6 with 3 N NaOH. Benzyl chloroformate (20 mL; 0.14 mol) was added dropwise with vigorous stirring. After 2.5 h the solution was washed twice with ethyl acetate, acidified to pH 2.2 with 6 N HCl, and extracted with three portions of ethyl acetate. The extracts were dried (Na_2SO_4) and concentrated. The solution was heated on the steam bath, treated with hexanes to a faint turbidity, and allowed to crystallize to yield the product as a white powder: 23.3 g (84.3%); mp 111–112 °C; ^1H NMR ($CDCl_3$) δ 1.1–1.9 (br m, 6 H), 3.42 (t, 2 H), 3.95 (m, 1 H), 5.08 (s, 2 H), 7.44 (s, 5 H), overlapping with 7.56 (d, 1 H, NH).

(D,L)-*N*-Cbz-ε-Hydroxynorleucine Benzyl Ester (7a). (D,L)-*N*-Cbz-ε-Hydroxynorleucine (5.0 g, 17.8 mmol) was suspended in CH_3CN (40 mL) and treated with *O*-benzyl-*N,N'*-diisopropylisourea[56] (5.0 g, 21.3 mmol). The mixture was stirred at room temperature for 18 h, filtered, and evaporated. The residue was chromatographed on silica gel (50 ×

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(55) Friedlander, *Fortschr. Teerfarb. Fabrik.* **1907**, *8*, 958.
(56) Mathias, L. J.; Fuller, W.; Nissen, D.; Goodman, M. *Macromolecules* **1978**, *11*, 534.

4 cm), eluting with CH_2Cl_2–*i*-PrOH (97:3) to yield an oil: 5.7 g (86%); ^1H NMR ($CDCl_3$) δ 1.1–1.9 (br m, 6 H), 1.99 (s, 1 H, OH), 3.51 (t, 2 H), 4.35 (m, 1 H), 5.07 (s, 2 H), 5.13 (s, 2 H), 5.5 (d, 1 H), 7.33 (s, 10 H).

Anal. Calcd for $C_{21}H_{25}NO_5$: C, 67.90; H, 6.78; N, 3.77. Found: C, 67.53; H, 6.78; N, 3.94.

(L)-*N*-Boc-ε-Hydroxynorleucine (6b). We previously reported this compound in a communication;[35] the synthetic details are given here. (L)-ε-Hydroxynorleucine (8.5 g, 0.060 mol) was dissolved in THF–H_2O (1:1, 300 mL) and treated with Et_3N (8.4 mL; 0.060 mol) followed by di-*tert*-butyl dicarbonate (15.72 g, 0.072 mol) in THF (20 mL). The solution was stirred at room temperature overnight. After removal of THF, 1 N NaOH (75 mL) was added and the solution was washed with two portions of ethyl acetate. The solution was acidified to pH 3.0 with solid citric acid and extracted with three portions of ethyl acetate. The extracts were dried (Na_2SO_4) and evaporated to leave a crystalline solid (13.7 g, 96%), which was recrystallized from ethyl acetate–hexanes to yield **6b**: 11.4 g (80%); mp 112–113 °C; $[\alpha]^{24}_D$ −6.36 ± 0.8 (*c* 7.3, MeOH); ^1H NMR ($CDCl_3$) δ 1.2–2.0 (m, 15 H, includes *tert*-butyl singlet at 1.46), 3.66 (t, 2 H), 4.2 (m, 1 H), 5.2–5.6 (br s , 1 H, NH), 7.51 (s, 2 H, OH/COOH).

Anal. Calcd for $C_{11}H_{21}NO_5$: C, 53.42; H, 8.56; N, 5.67. Found: C, 53.37; H, 8.46; N, 5.67.

(D,L)-*N*-Boc-ε-Hydroxynorleucine. This was prepared in the same manner as above from (D,L)-ε-hydroxynorleucine. The product had identical spectral properties, mp 94–98 °C.

(D,L)-*N*-Boc-ε-Hydroxynorleucine Methyl Ester (7b). (D,L)-*N*-Boc-ε-Hydroxynorleucine (7.03 g, 0.0284 mol) was dissolved in CH_2Cl_2 (30 mL) and treated with *O*-methyl-*N,N'*-diisopropylisourea (4.50 g, 0.0284 mol). The solution was refluxed for 20 h. The reaction mixture was chilled and filtered (to remove most of the *N,N'*-diisopropylurea) and then chromatographed on silica gel (4 × 50 cm), eluting with CH_2Cl_2–MeOH (95:5). The product was an oil of which 2.35 g (32%) was essentially pure and a further 2.5 g (34%) was somewhat contaminated. When chromatography was deleted, the yield was estimated to be about 90% and the product was suitable for further use. ^1H NMR ($CDCl_3$) δ 1.2–1.9 (br m, 15 H, includes *tert*-butyl singlet at 1.46), 2.23 (br s, 1 H, OH), 3.66 (br t, 2 H), 3.74 (s, 3 H), 4.0–4.4 (br m, 1 H), 5.18 (d, 1 H).

***O*-Benzyl acetohydroxamate (8)** was prepared by the method of Nicolaus et al.[51] except that the product was purified by acid–base extraction rather than by vacuum distillation.

***O*-Benzyl-*N*-Cbz-hydroxylamine (9).** *O*-Benzylhydroxylamine–HCl (3.19 g, 0.02 mol) was suspended in dry acetonitrile (40 mL) and treated with pyridine (3.23 mL, 0.04 mol). After the mixture was cooled to 0 °C, benzyl chloroformate (2.85 mL, 0.02 mol) was added dropwise with stirring. The mixture was allowed to warm up to room temperature and vigorously stirred for 24 h. Volatile components were evaporated, and the residue was taken into ethyl acetate. This was washed twice with 0.6 N HCl, once with H_2O, once with 0.6 M $NaHCO_3$, and once again with H_2O. After the mixture was dried ($MgSO_4$) and evaporated, the residue (5.1 g) was crystallized from ethyl acetate–hexanes to yield **9** as colorless crystals: 3.09 g (60%), mp 65.6–66.8 °C (lit. mp 65–68 °C)[57]; ^1H NMR ($CDCl_3$) δ 4.8 (s, 2 H), 5.12 (s, 2 H), 7.32 (s, 10 H), 7.63 (s, 1 H, NH).

General Procedure for Alkylation of Hydroxamates 8 and 9 with Alcohols 7a,b Mediated by PPh_3–DEAD. The alcohol, **7a** or **7b**, as an approximately 0.1 M solution in dry THF, was treated with the hydroxamate, **8** or **9** (1.2 equiv), and PPh_3 (1.2 equiv). To this solution DEAD (1.2 equiv in a small amount of dry THF) was added dropwise over about 0.5 h with stirring at room temperature. The reactions were generally complete within an additional hour at room temperature, but if TLC or HPLC indicated the presence of the starting alcohol, the reaction was left overnight or treated with slightly more PPh_3 and DEAD. Products were characterized by isolation and spectral and elemental analyses when possible, but in the alkylations of *O*-benzyl acetohydroxamate some products were characterized by TLC and HPLC comparison with products described below. Chromatography on silica gel was the standard method of isolation.

When *O*-benzyl acetohydroxamate was alkylated by the general procedure with **7a**, the products **10a–12a** were detected by TLC and HPLC comparison with the same compounds prepared as described below. In addition, small amounts of **11a** and **13a** were obtained pure by chromatography, eluting with hexanes–ethyl acetate (70:30) to obtain two fractions, which were each rechromatographed, eluting with CH_2Cl_2–ethyl acetate (90:10). **11a** was recrystallized from benzene–hexanes: mp 75–77 °C; ^1H NMR ($CDCl_3$) δ 1.1–2.1 (br m, 6 H), 1.87 (s, 3 H), 3.86 (t, 2 H), 4.35 (m, 1 H), 4.91 (s, 2 H), 5.08 (s, 2 H), 5.13 (s, 2 H), 5.47 (d, 1 H, NH), 7.33 (s, 15 H). **13a** was obtained as an oil: ^1H NMR ($CDCl_3$) δ 1.0–2.0 (br m, superimposed with 6 sharp peaks centered at 1.20, 12 H), 3.46 (t, 2 H), 4.16 (m, 4 H), 4.2–4.5 (br m, 1 H), 5.09 (s, 2 H), 5.17 (s, 2 H), 5.48 (d, 1 H), 7.38 (s, 10 H).

When the carbamate derivative **9** was alkylated with **7a** by the general procedure, **14** was isolated in 62–70% yield by chromatography, eluting with CH_2Cl_2–*i*-PrOH (99.5:0.5). The product was a colorless oil: ^1H NMR ($CDCl_3$) δ 1.0–2.0 (br m, 6 H), 3.38 (t, 2 H), 4.1–4.5 (br m, 1 H), 4.81 (s, 2 H), 5.07 (s, 2 H), 5.12 (s, 2 H), 5.18 (s, 2 H), 5.36 (d, 1 H), 7.33 (s, 20 H).

Anal. Calcd for $C_{36}H_{38}N_2O_7$: C, 70.80; H, 6.27; N, 4.59. Found: C, 70.57; H, 6.08; N, 4.76.

(D,L)-*N*-Cbz-ε-Bromonorleucine Benzyl Ester (15a). (D,L)-*N*-Cbz-ε-Hydroxynorleucine benzyl ester (**7a**) (1.719 g, 4.63 mmol) and CBr_4 (2.301 g, 6.94 mmol) were dissolved in dry THF (30 mL) and treated dropwise with PPh_3 (1.821 g, 6.94 mmol) in THF (10 mL) such that the temperature did not rise much above ambient. After the mixture was stirred at room temperature for 12 h, the solvent was evaporated and the residue was chromatographed on silica gel (2 × 60 cm), eluting with CH_2Cl_2 to yield the bromide **15a** as a colorless oil: 1.778 g (89%); ^1H NMR ($CDCl_3$) δ 1.2–2.1 (m, 6 H), 3.29 (t, 2 H), 4.43 (m, 1 H), 5.09 (s, 2 H), 5.16 (s, 2 H), 5.36 (d, 1 H, NH), 7.36 (s, 10 H).

Anal. Calcd for $C_{21}H_{24}NO_4Br$: C, 58.07; H, 5.57; N, 3.23. Found: C, 57.78; H, 5.58; N, 3.48.

(L)-*N*-Boc-ε-Bromonorleucine Methyl Ester (15b). (L)-*N*-Boc-ε-Hydroxynorleucine (**6b**) (2.903 g, 11.7 mmol) was converted to the methyl ester as described for the racemic material, except that chromatography was deleted. After the *N,N'*-diisopropylurea was filtered off, the CH_2Cl_2 was removed and replaced with dry THF (30 mL). PPh_3 (4.208 g, 16 mmol) was added, followed dropwise by CBr_4 (5.468 g, 16 mmol) in THF (10 mL). After the mixture was stirred overnight at room temperature, the THF was removed and the residue was chromatographed on silica gel (4 × 50 cm), eluting with CH_2Cl_2 to yield a colorless oil: 2.508 g (66% for two steps); ^1H NMR ($CDCl_3$) δ 1.2–2.1 (br m, includes *tert*-butyl singlet at 1.43, 15 H), 3.40 (t, 2 H), 3.75 (s, 3 H), 4.27 (br m, 1 H) 5.07 (d, 1 H, NH); $[\alpha]^{24}_D$ −14.6 ± 1.2 (*c* 6.2, CH_3OH).

Anal. Calcd for $C_{12}H_{22}NO_4Br$: C, 44.45; H, 6.84; N, 4.32. Found: C, 44.29; H, 6.89; N, 4.20.

(D,L)-**15b** prepared in the same manner from (D,L)-**6b** had identical NMR and TLC properties.

(D,L)-N^2-Cbz-N^6-Acetyl-N^6-(benzyloxy)lysine Benzyl Ester (10a). (D,L)-*N*-Cbz-ε-Bromonorleucine benzyl ester (**15a**) (0.631 g, 1.46 mmol), *O*-benzyl acetohydroxamate (**8**) (0.349 g, 2.11 mmol), KI (0.1 g, 0.6 mmol), and anhydrous K_2CO_3 (0.8 g, 5.8 mmol) were placed in dry acetone (30 mL) and refluxed for 24 h. After the mixture was filtered and evaporated, the residue was chromatographed on silica gel (2 × 50 cm), eluting with CH_2Cl_2–ethyl acetate (92:8). The hydroxamate **10a** coeluted with a small amount of the excess *O*-benzyl acetohydroxamate, which was removed by washing the ethereal solution with 0.5 N NaOH and drying (alternatively the excess starting material can be washed out before chromatography). The product was obtained as a colorless oil: 0.492 g (65%); ^1H NMR ($CDCl_3$) δ 1.0–2.0 (br m, 6 H), 2.06 (s, 3 H), 3.56 (t, 2 H), 4.35 (br m, 1 H), 4.87 (s, 2 H), 5.09 (s, 2 H), 5.16 (s, 2 H), 5.2 (d, 1 H, NH), 7.35 and 7.39 (two s, total 15 H).

In addition, a small amount of the (*Z*)-hydroximate (**12a**) was obtained from the earlier column fraction (about 13%). This compound was more polar than the (*E*)-hydroximate (**11a**) isolated from the PPh_3–DEAD-mediated alkylations described above, would not crystallize, and differed slightly in ^1H NMR spectrum from **11a**. ^1H NMR ($CDCl_3$) δ 1.1–1.9 (br m, 6 H), 1.83 (s, 3 H), 3.93 (t, 2 H), 4.36 (br m, 1 H), 4.91 (s, 2 H), 5.07 (s, 2 H), 5.12 (s, 2 H), 5.5 (d, 1 H), 7.33 (s, 15 H).

When the catalytic KI was omitted and the reaction time was increased to 3 days, the yield of **10a** was the same, and the (*E*)-hydroximate (identical with that isolated from the PPh_3–DEAD-mediated alkylations) **11a** was obtained rather than the *Z* isomer, **12a**, and in about the same yield.

(L)-N^2-Boc-N^6-Acetyl-N^6-(benzyloxy)lysine Methyl Ester (10b). (L)-*N*-Boc-ε-Bromonorleucine methyl ester (**15b**) (1.854 g, 5.72 mmol), *O*-benzyl acetohydroxamate (**8**) (1.372 g, 8.30 mmol), KI (0.2 g, 1.2 mmol), and anhydrous K_2CO_3 (2.0 g, 14.5 mmol) were placed in dry acetone (25 mL) and refluxed for 24 h. The reaction mixture was filtered, evaporated, dissolved in ether and washed with 0.5 N NaOH to remove excess **8**. After the mixture was dried and evaporated, the residue was chromatographed on silica gel, eluting with CH_2Cl_2–ethyl acetate (92:8) until the O-alkylated material (apparently **12b**) was eluted and then with CH_2Cl_2–ethyl acetate (60:40) to obtain **10b** as a colorless oil: 1.536 g (66%); $[\alpha]^{25}_D$ −11.06 ± 1 (*c* 2.8, MeOH); ^1H NMR ($CDCl_3$) δ 1.1–2.0 (br m, includes *tert*-butyl singlet at 1.43, 15 H), 2.08 (s, 3 H), 3.62 (t, 2 H), 3.72 (s, 3 H), 4.2 (br, m, 1 H), 4.82 (s, 2 H), 5.12 (d, 1 H, NH), 7.39 (s, 5 H); mass spectrum, *m/e* 91 (100%, tropylium ion),

(57) Ludwig, B. J.; Dürsch, F.; Auerbach, M.; Tomeczek, K.; Berger, F. M. *J. Med. Chem.* **1967**, *10*, 556.

409 (3.1%, M + 1), 408 (0.5%, M^+).

Anal. Calcd for $C_{21}H_{32}N_2O_6$: C, 61.74; H, 7.90; N, 6.86. Found: C, 61.59; H, 7.88; N, 7.00.

(D,L)-**10b** prepared in the same manner from (D,L)-*N*-Boc-ε-bromonorleucine methyl ester had identical spectral, TLC, and HPLC properties.

(D,L)-N^6-Acetyl-N^6-hydroxylysine (2). (D,L)-N^2-Cbz-N^6-Acetyl-N^6-benzyloxylysine benzyl ester (**10a**) (0.240 g, 0.463 mmol) was dissolved in MeOH (10 mL), treated with 5% Pd on carbon (50 mg), and stirred under 1 atm of H_2 at room temperature for 4 h. After the second hour, H_2O (3 mL) was added to redissolve the precipitated product. The reaction mixture was filtered and evaporated, and the residue was crystallized from EtOH (aq) to give flaky white crystals: 84 mg (89%); mp 236–237 °C dec; $FeCl_3$ test for hydroxamic acids +++; ^1H NMR (D_2O) δ 1.1–2.1 (br m, 6 H), 2.0 (s, 3 H), 3.5 (m, 3 H); IR (KBr) 1580, 2950 cm^{-1}.

Anal. Calcd for $C_8H_{16}N_2O_4$: C, 47.04; H, 7.90; N, 13.72. Found: C, 47.42; H, 8.16; N, 14.00.

Hydrogenolysis of 14. When **14** was subjected to the hydrogenolysis conditions above, lysine (**16**) was obtained as shown by amino acid analysis of the reaction mixture.

N^2,N^2-(Anhydromethylene-1,5-citryl)bis[N^6-acetyl-N^6-(benzyloxy)-(L)-lysine methyl ester] (19). (L)-N^2-Boc-N^6-Acetyl-N^6-(benzyloxy)lysine methyl ester (**10b**) (0.678 g, 1.66 mmol) was stirred with CF_3CO_2H (2.5 mL) for 5 min at room temperature. Excess CF_3CO_2H was removed by rotary evaporation. The residue was partitioned between $CHCl_3$ (25 mL) and 1 M Na_2CO_3. The basic chloroform layer containing the free amine, **17**, was briefly dried (K_2CO_3) and chilled to 0 °C. Et_3N (0.231 mL, 1.66 mmol) was added, followed by the slow dropwise addition of anhydromethylenecitryl chloride[55] (**18**) (0.200 g, 0.83 mmol) in $CHCl_3$ (5 mL). After the solution was stirred 5 min at 0 °C, it was warmed to room temperature and stirred an additional 40 min. Next the solution was washed with 0.5 M citric acid (aq), H_2O, 0.5 M $NaHCO_3$, and finally with brine. Finally it was dried ($MgSO_4$) and evaporated to leave a colorless glass: 0.540 g (83%); $[\alpha]^{27}_D$ −13.62 ± 1 (*c* 2.4, MeOH); ^1H NMR ($CDCl_3$) δ 1.1–1.9 (m, 12 H), 2.10 (s, 6 H), 2.83 (s, 4 H), 3.63 (t, 4 H), 3.71 (s, 6 H), 4.49 (m, 2 H), 4.82 (s, 4 H), 5.51 (s, 2 H), 6.92 (br d, 2 H), 7.38 (s, 10 H); mass spectrum, *m/e* 91 (100%, tropylium ion), 785 (10.7%, M + 1), 784 (1.5%, M^+).

N^2,N^2-(1,5-Citryl)bis[N^6-acetyl-N^6-(benzyloxy)-(L)-lysine] (20). Compound **19** (0.354 g, 0.451 mmol) was dissolved in THF–H_2O (1:1, 25 mL), treated with 1 N NaOH (1.36 mL), and stirred at room temperature for 2 h. The reaction mixture was passed through a column of Dowex-50 X-8 (H^+), 25-mL bed, and washed through with a further 60 mL of the same solvent. The sodium-free solution was evaporated at reduced pressure to yield a brittle glass: 0.331 g (98.5%); $[\alpha]^{23}_D$ +12.43 ± 2 (*c* 2, MeOH); ^1H NMR ($CDCl_3$) δ 1.0–2.0 (m, 12 H), 2.08 (s, 6 H), 2.82 (s, 4 H), 3.61 (t, 4 H), 4.5 (m, 2 H), 4.80 (s, 4 H), 7.36 (s, 10 H), 7.92 (d, 2 H); mass spectrum, *m/e* 745 (100%, M + 1), 91 (2%, tropylium ion) .

(−)-Aerobactin (1). Compound **20** (183 mg, 0.246 mmol) was dissolved in MeOH (6 mL) and treated with 5% Pd on carbon (60 mg). The mixture was stirred at room temperature under 1 atm of H_2 for 4 h and then filtered and evaporated. The residue was taken into H_2O (5 mL), Millipore filtered, and lyophilyzed to yield **1** as a hygroscopic, slightly off-white powder, 125 mg (90%). The 100-MHz ^1H (D_2O) and IR spectra (KBr) were identical with those depicted in the literature for natural aerobactin.[31] Paper chromatography (concentrated NH_3–EtOH–H_2O (1:16:3) and *n*-BuOH–HOAc–H_2O (60:15:25)) gave the same R_f values (0.14 and 0.61) as reported for the natural substance (0.13 and 0.63).[31] $[\alpha]^{22}_D$ −10.83 ± 1.4 (*c* 1.7, H_2O); mass spectrum, *m/e* 565 (100%, M + 1); no literature data available.

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Registry No. 1, 26198-65-2; **2**, 81505-47-7; DL-**5**, 5462-80-6; L-**5**, 6033-32-5; **6a**, 32245-65-1; **6b**, 77611-37-1; **7a**, 81505-48-8; **7b**, 81505-49-9; **8**, 4797-81-3; **9**, 15255-86-4; **10a**, 81505-50-2; **10b**, 81505-51-3; **11a**, 81505-52-4; **11b**, 81505-53-5; **12a**, 81505-54-6; **12b**, 81505-55-7; **13a**, 81505-56-8; **14**, 81505-57-9; **15a**, 81505-58-0; **15b**, 81505-59-1; **16**, 56-87-1; **17**, 81505-60-4; **18**, 81505-61-5; **19**, 81505-62-6; **20**, 81505-63-7; DL-*N*-Boc-ε-hydroxynorleucine, 81505-64-8; *O*-benzylhydroxylamine HCl, 2687-43-6.

Guanosine 5′-Monophosphate Catalyzed Hydrolysis of Diastereomeric Benzo[*a*]pyrene-7,8-diol 9,10-Epoxides

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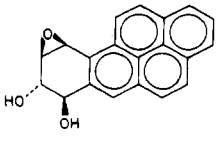
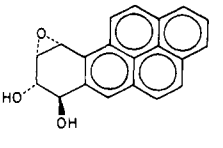
Contribution from the Laboratory for Chemical Dynamics, Deparment of Chemistry, University of Maryland Baltimore County, Baltimore, Maryland 21228, and the Laboratory of Bioorganic Chemistry, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205. *Received August 10, 1981*

Abstract: The rates of reaction of 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene and 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene in aqueous dioxane solutions in the presence of 5′-guanosine monophosphate (5′-GMP) and guanosine (G) have been determined. 5′-GMP in the monohydrogen form ($GMPH^-$) exhibits pronounced general acid catalysis in the hydrolysis of these epoxides under conditions where the corresponding nucleoside G is unreactive. Although the pK_a values for $GMPH^-$ and inorganic dihydrogen phosphate ion ($H_2PO_4^-$) are very similar, $GMPH^-$ is 60–80 times more efficient than $H_2PO_4^-$ in catalyzing the hydrolysis of the diol epoxides. Significant solvent effects on both $GMPH^-$ and $H_2PO_4^-$ have been observed.

The metabolic activation of the environmental carcinogen benzo[a]pyrene leads in part to the two diastereomeric 7,8-diol 9,10-epoxides **1** and **2**.[2a] The mutagenic and carcinogenic



1



2

properties of these highly reactive metabolites have been attributed to their covalent binding to cellular macromolecules.[2b–f] The major products from covalent binding of **1** and **2** to DNA result from

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(2) (a) The complete names of **1** and **2** are (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene and (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, respectively. For reviews, see: (b) Miller, E. C. *Cancer Res.* **1978**, *38*, 1479. (c) Levin, W.; Wood, A. W.; Wislocki, P. G.; Chang, R. L.; Kapitulnik, J.; Mah, H. D.; Yagi, H.; Jerina, D. M.; Conney, A. H. In "Polycyclic Hydrocarbons and Cancer"; Gelboin, H. V., T'so, P. O., Eds.; Academic Press: New York, 1978, Vol 1, p 189. (d) Yang, S. K.; Deutsch, J.; Gelboin, H. V. *Ibid.* Vol. 1, p 205. (e) Gelboin, H. V. *Physiol. Rev.* **1980**, *60*, 1107. (f) Harvey, R. G. *Acc. Chem. Res.* **1981**, *14*, 218.

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